PRESENCE OF ERABUTOXINS A AND B IN VENOM OF THE SEA SNAKE LATICAUDA SEMI-FASCIATA FROM TAIWAN

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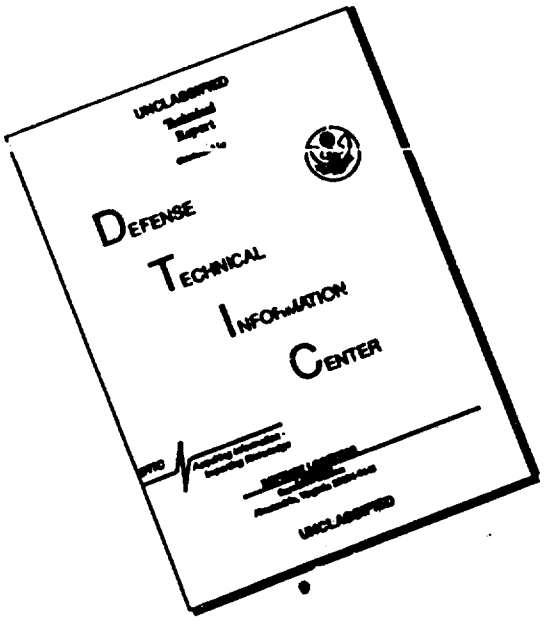

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SHORT COMMUNICATION

PRESENCE OF ERABUTOXINS a AND b IN VENOM OF THE SEA SNAKE *LATICAUDA SEMIFASCIATA* FROM TAIWAN*

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TWO PRINCIPAL toxins from venom of the sea snake *Laticauda semifasciata* have been purified and their amino acid compositions studied previously. TAMIYA and ARAI (1966), working with venom obtained from sea snakes collected in Okinawan waters, called the two toxins Erabutoxins a and b; amino acid compositions and sequences of the two compounds were reported by SATO and TAMIYA (1971). TU *et al.* (1971) obtained two similar toxins, called Toxins a and b, from the same species of *Laticauda* collected in Philippine waters; results of their amino acid analyses are somewhat different from those of Sato and Tamiya. In the present paper we report results of studies made on the two principal toxins obtained from venom of *Laticauda semifasciata* collected in Taiwan. As summarized below, our results are in complete agreement with those of Sato and Tamiya.

Sea snakes used in this study were collected in the vicinity of Pescadores Islands in the Formosan Strait west of Taiwan and near Green Island and Orchid Island which are near the southeast end of Taiwan. Venom was collected by milking live snakes and was pooled for analysis. Methods used for venom fractionation and further chemical characterizations were essentially those reported in our previous study of *Hydrophis cyanocinctus* (LIU *et al.*, 1973).

Protein fractions obtained by chromatographic separation of venom components are shown in Fig. 1. Fractions IV, V and VI had relatively high lethality as measured by screening tests in mice. For example, 10 μg quantities of each fraction, injected intraperitoneally (in 0·2 ml of saline) into 20 g mice caused death in all animals within 30 min; five mice were used to test each fraction at the 10 μg level. Higher amounts of all three fractions also killed all animals tested, while studies were not made at lower levels.

Fraction IV was not investigated further; from its chromatographic behavior, it appears to be identical with the minor fraction, Erabutoxin c, described by TAMIYA (1972) and TAMIYA and ABE (1972). Fractions V and VI were desalted with a Sephadex G-15 column and purified by column chromatography on CM 52 carboxymethylcellulose using an ammonium acetate buffer system. Portions of purified fractions were lyophilized, carboxy-

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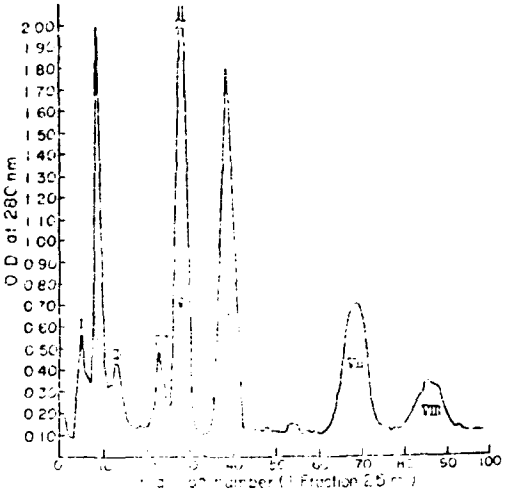

FIG. 1. RESULTS OF TYPICAL SEPARATION OF PROTEIN COMPONENTS FROM LYOPHILIZED VENOM OF *Laticauda semifasciata*.

Seventy-seven milligrams of dry venom were dissolved in 2 ml of the initial buffer (pH 6·3, 0·01 M sodium phosphate containing 0·01 M sodium chloride) and applied to the column packed with Whatman carboxymethylcellulose, CM-52. Gradient elution was achieved with 500 ml of initial buffer and 500 ml of final buffer (pH 6·3, 0·01 M sodium phosphate containing 0·5 M sodium chloride). Fractions V and VI were isolated for further study.

TABLE 1. COMPARISON OF AMINO ACID COMPOSITIONS OF TOXINS FROM *Laticauda semifasciata*

Amino acid residue	Fraction V nmoles	Fraction V Molar ratio	Erabutoxin a Molar ratio*	Toxin a Molar ratio†	Fraction VI nmoles	Fraction VI Molar ratio	Erabutoxin b Molar ratio*	Toxin b Molar ratio†
CM-Cys	215	8·0	8	8	150	8·0	8	8
Asp	135	5·0	5	5	75	4·0	4	4
Thr	125	4·6	5	6	90	4·8	5	5
Ser	205	7·6	8	7	145	7·7	8	6
Glu	225	8·3	8	8	150	8·0	8	8
Pro	90	3·3	4	4	65	3·5	4	4
Gly	140	5·2	5	5	95	5·1	5	6
Val	50	1·9	2	2	34	1·8	2	3
Ile	80	3·0	4	4	55	3·0	4	4
Leu	27	1·0	1	1	16	0·9	1	1
Tyr	32	1·2	1	1	17	0·9	1	1
Phe	55	2·0	2	2	33	1·8	2	2
Lys	110	4·1	4	4	75	4·0	4	5
His	32	1·2	1	1	42	2·2	2	1
Arg	80	3·0	3	3	50	2·7	3	2

* SATO and TAMIYA (1971).
† TU *et al.* (1971).

methylated, hydrolyzed for 24 hr at 108°C with constant boiling hydrochloric acid containing mercaptoethanol (1/2000 v/v) (KEUTMANN and POTTS, 1969), and analyzed for their amino acid composition. Results are given in Table 1 and compared with results obtained

by previous workers. Except for isoleucine, where recovery was incomplete, presumably because of incomplete hydrolysis of the isoleucylisoleucine bond (SATO and TAMIYA, 1971), amino acid compositions of Fractions V and VI agree completely with those given by SATO and TAMIYA (1971) for Erabutoxins a and b. Compositions of Toxins a and b reported by TU *et al.* (1971) had several differences.

Peptide maps of our tryptic digests of Fractions V and VI were identical except for one peptide from each which had different mobilities. Those peptides were analyzed for amino acid composition with the following results. The peptide from Fraction V had 2 cysteinyl and 3 seryl residues and one each of asparaginyl (determined as aspartyl after hydrolysis), threonyl, glutamyl, prolyl, glycyl, tyrosyl, and lysyl. Composition of the peptide from Fraction VI was the same except for absence of asparaginyl and presence of one histidyl group. These results match those obtained by SATO and TAMIYA (1971) for that section of the erabutoxin molecules between amino acid residues 16 and 27. The sole difference between Erabutoxins a and b reported by SATO and TAMIYA (1971) was at position 26 which was occupied by asparaginyl in Erabutoxin a and by histidyl in Erabutoxin b.

From these results it was concluded that the two toxic fractions isolated from venoms collected from specimens of *Laticauda semifasciata* in Taiwan are identical to those reported by Sato and Tamiya in *L. semifasciata* from Okinawa and different from Toxins a and b reported by TU *et al.* (1971) from *L. semifasciata* from the Philippines.

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